cartilage extracts. The dog ACLT model of acute injury showed higher levels of TN-C in surgery knees at 1-month post surgery (73-fold) and 3-months post surgery (48-fold) as compared to control knees. Higher levels of TN-C were maintained in surgery knees 6-months post surgery in this dog model. In the rat meniscal tear model, there was a significant increase (40-fold) in TN-C in surgery knees at 1wk as compared to no surgery contralateral controls, and this increase was maintained at 4 and 8 wks, albeit with a smaller absolute difference from control.

Conclusions: The potential of TN-C Large as a unique biomarker of joint disease/injury has been demonstrated using synovial fluids from humans with various joint diseases and from preclinical animal models of joint injury. Being elastic, TN-C might play an important role in degenerative/regenerative processes where the normal biomechanical environment of musculoskeletal tissue is compromised by disease/injury. As a binder of several ECM proteins, release of TN-C could have a larger impact on the integral structure/function of other ECM proteins, and has the potential to be a marker of joint pathobiology and healing. Our preliminary results indicate that TN-C levels may be applicable to determining pharmacodynamic activity of chondro-protecting drugs in humans. Work is ongoing to study the levels of TN-C during degeneration in other joint tissues such as tendon. Understanding the functions of TN-C would provide insights for pharmacologic intervention of musculoskeletal diseases/injuries.

115

DEGRADATION TO SYNTHESIS RATIOS OF TYPE II COLLAGEN BIOMARKERS IN SYNOVIAL FLUID AND SERUM IN THOROUGHBRED RACEHORSES

T. Trumble[1], A. Nicholson[1], K. Merritt[2], M. Brown[2]
[1]Univ. of Minnesota, St. Paul, MN; [2]Univ. of Florida, Gainesville, FL

Purpose: Type II collagen biomarkers have shown promise in the study of osteoarthritis. CPII (cleaved C-propeptide of type II collagen) has been directly correlated with type II collagen synthesis. CTX II (crosslinked C-telopeptide fragments of type II collagen), C1,2C (neoepitope of types I and II collagen created after collagenase cleavage), and C2C (neoepitope of type II collagen created after collagenase cleavage) have been used to assess collagen degradation. The objective of the study was to compare type II collagen degradation to synthesis ratios in serum and synovial fluid (SF) from normal horses and those with osteochondral (OC) injury.

Methods: SF was taken from the carpal joints of 2 groups of Thoroughbred racehorses: (1) normal, adult horses > 3 years of age (2) OC injured horses 2-7 years of age undergoing arthroscopic surgery for removal of OC fragments resulting from racing injury. From group 1 horses, serum was collected from 16 horses. SF was obtained from 10 middle carpal joints (MCJ), and 10 radiocarpal joints (RCJ). From group 2, serum was collected from 20 horses. SF was collected from 10 MCJ and 10 RCJ. SF was aseptically collected by needle arthrocentesis without lavage, centrifuged, and decanted. Commercially available ELISAs, were used to measure type II collagen markers (C1,2C, C2C, CPII, and CTX II). Differences between each group were evaluated using an unpaired t-test. $P<0.05$ was considered significant.

Results: Concentrations of C2C; C1,2C; and CTX II were all significantly higher in SF from OC injured joints compared to normal joints (Table 1). Concentrations of CPII were also significantly higher in SF from injured joints compared to normal joints. Degradation to synthesis ratios in SF were significantly higher in OC injured carpal joints compared to normal joints for C1,2C:CPII and CTX II:CPII, but not for C2C:CPII. In serum, concentrations of C1,2C were significantly higher from OC injured horses compared to normal horses. Serum concentrations of CTX II were significantly lower from OC injured horses compared to normal horses. Serum ratios were significantly higher in horses with OC injured carpal joints compared to normal horses for C1,2C:CPII only. The ratio was significantly lower in serum from horses with OC injured carpal joints compared to normal horses for CTX II:CPII only.

Conclusions: Joint injury affects concentrations of type II collagen degradation and synthesis biomarkers and their ratios when compared to normal horses. In SF, C1,2C:CPII and CTX II:CPII ratios demonstrate that degradation predominates over synthesis when the joint is injured because the ratios are higher than normal joints. The serum C1,2C:CPII ratio suggests that there is higher degradation after injury compared to normal horses with no difference in the amount of synthesis. The CTX II:CPII ratio suggests that the synthesis of type II collagen stays steady with less degradation. However, increasing or decreasing degradation to synthesis ratios must be interpreted in light of the known effect of injury on biomarker concentrations in both SF and serum. Injury may cause increase in SF concentrations, but may at the same time cause an increase or decrease in serum concentrations. Thus, SF biomarkers may be more indicative of degradation or synthesis in a single joint than serum biomarkers.

116

SYNOVIAL FLUID URIC ACID AS A MARKER OF JOINT TISSUE DEGRADATION IN OSTEOARTHRITIS

T.V. Stabler, A. Heinrichs, G. McDaniel, K. Huffman, V.B. Kraus
Duke Univ. Med. Ctr., Durham, NC

Purpose: Uric acid (UA) is constitutively present in normal cells, increased in concentration when cells are injured and released from dying cells. The products of cell stress and tissue damage may represent "danger signals" that function as endogenous adjuvants recognized by the immune system. UA has been identified as one of these principal endogenous "danger signals" released from injured cells. We sought to determine whether elevated synovial fluid (SF) UA might be a potentiating factor in osteoarthritis (OA).

Methods: *Patients:* A total of 159 participants were enrolled in the Strategies to Predict Osteoarthritis Progression (POP) study. Informed consent was obtained from all subjects and the entire study was approved by the Duke University IRB. Participants met

Abstract 115 – Table 1. Mean (± SD) concentrations of serum and SF type II collagen biomarkers and degradation to synthesis ratios for normal and osteochondral (OC) injured carpal joints

	Mean biomarker concentrations (± SD)				Degradation:Synthesis		
	C2C (pmol/mL)	C1,2C (pmol/mL)	CTX II (pg/mL)	CPII (ng/mL)	C2C:CP II	C1,2C:CP II	CTX II:CP II
Serum							
Normal	479±32.2	**452±16*** **	**70.3±9.0** **	1045±91	0.43±0.19	**0.47±0.14***	**0.07±0.03** **
OC injured	415±9.8	**580±21.6**	**39.6±3.4**	1019±90	0.43±0.12	**0.63±0.21**	**0.04±0.01**
Synovial Fluid							
Normal	**274±15.6** **	**278±31.6*** **	**160±45*** **	**1013±60*** **	0.26±0.06	**0.22±0.05*** **	**0.08±0.05*** **
OC injured	**409±32.6**	**1070±122**	**513±47.1**	**1637±110**	0.26±0.09	**0.65±0.37**	**0.37±0.28**

Bolded values indicate a significant difference between groups within serum or SF. *P≤0.05; **P≤0.01; ***P≤0.001.

the ACR criteria for symptomatic OA of at least one knee and radiographic criteria for OA with a Kellgren-Lawrence (KL) score of 1-4 in at least one knee.
Radiographic Imaging: Posteroanterior semi-flexed knee radiographs were obtained and read for KL grade and individual radiographic features of OA, including joint space narrowing (JSN) and osteophytes (OST).
Scintigraphic Imaging: Scintigraphic images of the knee were obtained at 2 mins. (early phase) and 2.5 hrs. (late phase) after injection of technetium-99m methylene diphosphonate. The intensity of bone uptake was scored for the tibiofemoral and patellofemoral compartments of each knee.
Biochemical measurements: Matched serum and SF samples were analyzed for UA using HPLC. Cytokines were measured in the SF using the Bio-Rad human cytokine multiplex immunoassay for IL-5, IL-6, IL-7, IL-8, IL-13, MCP-1, and MIP-1 beta.
Statistical Analysis: Descriptive statistics and univariate analyses were performed using Graphpad Prism software. Relationships between SF analytes and OA were analyzed using the GenMOD procedure with the addition of a repeated statement (GLM, SAS Enterprise Guide). Multivariate modeling was used to assess independent effects of SF analytes and to control for BMI, age, and gender.
Results: This study was limited to 69 study participants (49 women and 20 men) with knee OA and adequate SF volume for these analyses. The mean (±SD) age was 64.5±10.1 years. The mean (±SD) body mass index was 32.4±7.1 kg/m^2. Knee OA was graded 1-4 in severity (23.1%, 14.6%, 49.2%, 13.1% for each KL grade). SF measurements were possible for both knees of 63 participants, and for single knees of 6 participants.
The mean UA (±SD) was 6.43±1.75 mg/dl for serum, 5.17±1.79 mg/dl for the right knee SF, and 4.93±1.95 mg/dl for the left knee SF. Serum UA was highly correlated with SF UA ($p<0.0001$), but UA was consistently higher in serum in nearly all individuals ($p<0.0001$).
SF UA was associated with increased SF IL-8, a marker of inflammation associated with macrophage activation ($p<0.0001$), but did not correlate with other markers in the cytokine panel.
SF, but not serum, UA was positively associated with all three measures of OA severity.

Relationship between analytes and Knee OA severity (P values)

	Late Phase Bone Scan*		JSN		OST		JSN + OST	
	Total	TF	Total	TF	Total	TF	Total	TF
SF UA	0.04	0.03	0.29	0.05	0.01	0.0003	0.02	0.001
SF lnIL-8	0.06	0.01	0.04	0.007	0.0001	<0.0001	0.002	<0.0001
Serum UA	0.02	0.04	0.08	0.33	0.83	0.63	0.53	0.50

*Early Phase Bone Scan controlled for in model. Total = Tibiofemoral (TF) + Patellofemoral. JSN = Joint Space Narrowing. OST = Osteophytes.

Multivariate Generalized Linear Modeling (P values)

	Outcomes							
	Late Phase Bone Scan*		JSN		OST		JSN + OST	
	Total	TF	Total	TF	Total	TF	Total	TF
SF UA	0.02	0.02	0.34	0.06	0.02	0.0006	0.04	0.002
Early Phase	<0.0001	<0.0001						
BMI	<0.0001	0.005	0.15	0.03	0.58	0.58	0.42	0.29
Age	0.03	0.21	0.08	0.13	0.53	0.26	0.33	0.19
Gender	0.41	0.98	0.21	0.17	0.87	0.40	0.83	0.28

*Early Phase Bone Scan controlled for in model. Total = Tibiofemoral (TF) + Patellofemoral. JSN = Joint Space Narrowing. OST = Osteophytes.

Conclusions: The strong association shown here between OA severity, particularly osteophytes, and SF UA, demonstrates that UA is a marker of joint tissue injury and raises the strong possibility that UA may be a factor contributing to the pathological process of OA. The constitutive release of UA from injured and dying cells in the OA joint may exacerbate joint tissue inflammation through sub-acute macrophage activation. Urate crystallization in the presence of nucleating agents released from degrading cartilage extracellular matrix may also contribute to joint tissue inflammation.

117

THE RELATIONSHIP BETWEEN RADIOLOGICAL GRADE OF KNEE OSTEOARTHRITIS AND BIOCHEMICAL MARKERS OF CARTILAGE AND BONE DEGRADATION (URINE CTX-II AND NTX-I): THE MATSUDAI KNEE OSTEOARTHRITIS SURVEY 2007

H. Yamagiwa[1], T. Hayami[2], G. Omori[3], N. Tanishi[1], H. Mera[1], Y. Koga[4], N. Endo[1]
[1]Dept. of Orthopedic Surgery., Niigata Univ., Niigata, Japan; [2]Dept. of Orthopedic Surgery, Saiseikai Niigata Daini Hosp., Niigata, Japan; [3]Ctr. for Transdisciplinary Res., Niigata Univ., Niigata, Japan; [4]Dept. of Orthopedic Surgery, Niigata Kobari Hosp., Niigata, Japan

Purpose: Biochemical markers of cartilage and bone degradation are becoming increasingly important in the evaluation of knee Osteoarthritis (OA). The correlation between radiological knee OA and urine CTX-II (C-terminal crosslinking telopeptide of collagen type II) or urine NTX-I (N-terminal crosslinking telopeptide of type I collagen) needs to be evaluated how these markers are useful in the health check-up in a large population-based study.
Methods: We have performed the epidemiological knee survey in a rural Japanese population every 7 years at the Matsudai district in Niigata Prefecture, Japan since 1979. In 2007 (5th survey), a cross-sectional study of biomarkers was conducted with informed consent in the historical cohorts. Urine specimens were collected from 1040 subjects (605 females and 435 males), and CTX-II and NTX-I were measured using ELISA. Menstruation status and oral administration of bisphosphonate were checked. Standing knee AP X-rays were obtained and graded according to the Kellgren-Lawrence (K-L) classification. The subjects were then divided by gender, age (40- to 59-year-old group and 60- to 79-year-old group), and the X-ray grade (K-L Grade 0,1, Grade 2, and Grade 3,4). The values of CTX-II and NTX-I were compared between age groups and OA grade groups. Mann Whitney U test, Kruskal-Wallis test, and Spearman's rank correlation test were used, and $p<0.05$ was considered statistically significant.
Results: In non-OA (Grade 0,1) subjects, there was no significant difference in CTX-II values between two age groups in males. However, in female, the 40- to 59-year-old postmenopausal group and the 60- to 79-year-old group had significantly higher CTX-II values than the 40- to 59-year-old premenopausal group. Administration of bisphosphonate also affected strongly both CTX-II and NTX-I values, so subjects taking bisphosphonate were excluded from further analysis.
In the male subjects aged over 60 years, knee OA Grade 3,4 group had significantly higher CTX-II values than the Grade 0, 1 group or the Grade 2 group (Fig. 1). In female subjects aged over 60 years, CTX-II values significantly increased according to the severity of knee OA (Fig. 1).
For NTX-I, there were no significant differences between each OA grade. However, weak but positive correlations were observed between the urine CTX-II and urine NTX-I values in the 40- to 59-year-old female group and the 60- to 79-year-old male and female groups (n=151, 352, 418, and rs=0.23, 0.10, 0.14, respectively).
Conclusions: Urine CTX-II can be a useful biomarker of knee OA stages even by the single measurement in people older than 60 years although it would be difficult for 40-59 year-old people to evaluate knee OA changes using single urine CTX-II values.